THE STRUCTURE OF SPHINGINE

BY

CHARLES GEORGE HUMISTON

B.S., Alma College, 1939

AN ABSTRACT OF A THESIS

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN CHEMISTRY
IN THE GRADUATE COLLEGE OF THE
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R. UNIV. BIBLIOTHEEK LEIDEN

THE STRUCTURE OF SPHINGINE

Introduction

Levene and West (1,2) reported the formation of an optically active base by the reduction of sphingosine. They found it to be an amino alcohol, which they called "sphingine", but did not further elucidate its structure.

Carter and associates (3) reduced triacetylsphingosine and found among the reduction products a substance whose properties resembled those of diacetylsphingine as prepared by Levene. It seemed desirable to develop a reproducible method for obtaining this material in sufficient quantity so that its structure might be determined. It appeared that oxidation of an N-acyl derivative of sphingine might yield an acylaminostearic acid which could be definitely characterized and compared with synthetic material. Moreover, if the aminostearic acid should be optically active, the determination of its configuration would establish the configuration of the amino carbon of sphingine, sphingosine and dihydrosphingosine.

Discussion Of Results

The amount of diacetylsphingine formed by the hydrogenation of triacetylsphingosine is dependent on the amount of hydrogenolysis taking place. The yield was largely dependent on the activity of the catalyst used. The best yield of diacetylsphingine was obtained by the hydrogenation of triacetylsphingosine in ethanol solution with freshly prepared platinum oxide as the catalyst.

Preferential saponification of the acetoxy group by treatment of diacetylsphingine with methanolic potassium hydroxide at room temperature formed N-acetylsphingine. This was converted to the free base by heating under reflux with five per cent potassium hydroxide in methanol. The free sphingine was converted to N-benzoylsphingine by treatment

of an alkaline solution of the base with benzoyl chloride. The formation of any dibenzoyl derivative was prevented by the addition of methanol. The diacetylsphingine, N-acetylsphingine, sphingine and N-benzoylsphingine were characterized by analyses, and their solubility behavior and optical properties were determined.

The N-benzoylsphingine was oxidized by heating a glacial acetic acid solution under reflux for thirty minutes with chromic acid. An optically active (—)α-benzamidostearic acid was formed. This compound was identified by racemization through formation of the azlactone, subsequent hydrolysis and comparison of the racemized product with a synthetic sample of α-benzamidostearic acid.

Neuberger (4) has reviewed the various methods used in the past for establishing the configuration of α-amino acids and other optically active compounds. Similarities in behavior under various conditions have been used to indicate configurational relationships. In an attempt to determine the configuration of the (—)α-benzamidostearic acid formed from N-benzoylsphingine, the change in optical rotation in dioxane and acetic acid solutions with increasing amounts of water present was studied. Model studies were made using various acylamino acids in these same solvents. The L-α-amino acid derivatives studied were: benzoyl-L-alanine, β-phenylpropionyl-L-leucine, β-phenylpropionyl-L-phenylalanine and p-toluenesulfonyl-p-methoxy-L-phenylalanine. All of these compounds in both dioxane and acetic acid solutions showed an increase in levorotation when increasing amounts of water were present. Formyl-D-isoleucine in dioxane and acetic acid solutions showed a decrease in levorotation as the water content was increased. The active α-benzamidostearic acid showed a decrease in levorotation under these same conditions. From these results, the (—)-α-benzamidostearic acid derived from sphingosine appears to have the D configuration. Other criteria will have to be tested before the configuration is definitely established.

Summary

A reproducible method has been developed for the preparation of diacetylsphingine by the hydrogenolysis and reduction of triacetylsphingosine in ethanol solution using platinum oxide as the catalyst.

N-Acetylsphingine, sphingine, and N-benzoylsphingine have been prepared and characterized by analysis, solubility behavior and optical properties.

A method is described for the oxidation of N-benzoylsphingine to the corresponding optically active α-benzamidostearic acid in good yield. This compound was racemized by conversion to the azlactone, subsequently hydrolyzed and compared with synthetic material.

A study was made of the optical rotation of a number of acylamino acids in organic solvents containing varying amounts of water. The derivatives of L-amino acids in dioxane and acetic acid solutions showed an increase in levorotation when increasing amounts of water were present. Since solutions of (—)-α-benzamidostearic acid in these solvents showed a decrease in levorotation on dilution with water, it seems probable that this compound has the D-configuration.

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VITA

The author was born on February 2, 1919, in Lakeview, Michigan. After graduating from Clawson High School, Clawson, Michigan in May 1935, he entered Alma College the following September and received the degree of Bachelor of Science, with honors, from that institution in June, 1939. He began graduate studies in chemistry at Oberlin College where he held a half-time graduate assistantship in chemistry from 1939 to 1941. He continued graduate studies at Michigan State College where he served as half-time graduate assistant in biochemistry for two semesters. In 1942 he enlisted in the Army of the United States and served in the American and European Theaters. Upon discharge from the Army in 1946, he entered the Graduate School of the University of Illinois. He held a Special Research Assistantship from September, 1947 to June, 1948, and an Upjohn Company Research Fellowship from October, 1948 to August, 1949.

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